Kidney International, Vol. 60 (2001), pp. 31–36

GENETIC DISORDERS – DEVELOPMENT

Nephron endowment in glial cell line-derived neurotrophic factor (GDNF) heterozygous mice

LUISE A. CULLEN-MCEWEN, JOHN DRAGO, and JOHN F. BERTRAM

Department of Anatomy and Cell Biology, Department of Medicine, Monash University, Clayton, Victoria, Australia

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Background. The exact molecular mechanisms that regulate ureteric branching morphogenesis in the developing metanephros have not been fully elucidated. However, in vivo and in vitro evidence indicates that glial cell line-derived neurotrophic factor (GDNF) is a key regulator of the initiation of ureteric branching. GDNF knockout mice show renal agenesis or severe dysgenesis and die 24 hours after birth from renal failure. Inhibition of GDNF activity in metanephric organ culture inhibits ureteric branching. Since nephron initiation only occurs at the tips of ureteric branches, the aim of the present study was to determine whether nephron number in GDNF heterozygous mice is reduced.

Methods. Male GDNF heterozygous mice of hybrid 129/*Sv* and C57/BL genetic background were mated with C57BL/6 females. Offspring were genotyped at postnatal day 30 (PN30) by polymerase chain reaction. Left kidneys were used for estimating kidney volume and total nephron number. We also estimated absolute and relative volumes of ureteric duct epithelium. Unbiased stereological methods were used throughout (Cavalieri method, physical disector/fractionator combination).

Results. GDNF wild-type and heterozygous mice had similar body weights at PN30. However, heterozygous kidneys were 25% smaller than wild-type kidneys (wild-type, 114.75 ± 16.46 mm^3; heterozygous, 87.11 ± 21.84 mm^3, $P < 0.001$) and contained approximately 30% fewer nephrons (wild-type, 11886 ± 1277; heterozygous, 8573 ± 2240, $P < 0.01$). In addition, the absolute ureteric duct volume was significantly reduced in heterozygous mice ($P < 0.001$).

Conclusions. These results indicate that the loss of one GDNF allele results in reduced nephron endowment in the adult kidney, presumably as the result of reduced branching morphogenesis of the ureteric bud.

The development of the permanent kidney (metanephros) is characterized by a series of inductive interactions between the epithelial ureteric bud and the metanephric mesenchyme. The mesenchymal cells induce growth and branching of the ureteric bud, giving rise to the collecting duct system. Simultaneously, the tips of the branching duct induce the surrounding mesenchyme to condense, epithelialize, and differentiate into nephrons.

Until 1996, very little was known about the molecular regulation of ureteric branching morphogenesis. Glial cell line-derived neurotrophic factor (GDNF), a distant member of the transforming growth factor-β superfamily, mediates its signal via the receptor tyrosine kinase, rearranged during transfection (RET), and the coreceptor, GDNF family receptor α1 (GFRα1). A role for GDNF in ureteric branching morphogenesis was suggested by its restricted expression pattern and by the expression patterns of RET and GFRα1. GDNF mRNA is observed in uninduced mesenchyme, is strongly up-regulated in pretubular condensates, and is rapidly down-regulated with the transition to epithelium [1, 2]. In contrast, RET mRNA is initially expressed in the body of the ureteric epithelium but is later restricted to the tips of the branching ureteric buds [3]. GFRα1 mRNA is found at high levels in the ureteric buds and in adjacent mesenchyme [4].

Mice lacking RET [5, 6], GDNF [7–10], or GFRα1 [11, 12] all die within 24 hours of birth from renal failure. Each homozygous mutant shares similar phenotypes of kidney agenesis or severe dysgenesis. Each of the heterozygous mutants are viable and fertile. However, macroscopic examination of adult GDNF heterozygous mutants reveals a wide range of renal abnormalities, including small kidneys, abnormal shape, cortical cysts, unilateral agenesis, or severe dysgenesis [8, 9]. The kidneys of RET and GFRα1 heterozygous mutant mice, however, appear normal [5, 11, 12].

Histologic analysis of GDNF mutant mice revealed that frequently the ureter fails to bud from the Wolffian duct even though morphologically distinct metanephric mesenchyme is present. In animals in which the ureter did bud and penetrate the mesenchyme, the number of collecting ducts, condensates, and nephrons was severely reduced. These defects were also observed in some heterozygous embryos [8, 9]. The observed phenotypes cor-

Key words: kidney development, nephron endowment, metanephros, stereology, ureteric branching morphogenesis.

Received for publication November 6, 2000
and in revised form January 18, 2001
Accepted for publication January 23, 2001

responded with alterations to branching of the ureteric duct epithelium. Several in vitro studies have since provided a link between GDNF and ureteric branching morphogenesis [4, 13–15].

It is now generally accepted that GDNF is the first factor identified to regulate in a dose-dependent manner the growth and arborization of the ureteric bud during early stages of kidney morphogenesis. The results of the knockout studies indicate that GDNF gene dosage influences kidney development, with the loss of one allele being sufficient to cause a significant renal phenotype.

The aim of the present project was to determine whether the loss of one GDNF allele results in the development of kidneys with reduced numbers of nephrons. We estimated the total nephron number in kidneys of postnatal 30 days (PN30), GDNF heterozygous null-mutant mice and wild-type mice. We also estimated the absolute and relative volumes of the ureteric duct epithelium in heterozygous and wild-type mice.

METHODS

Animals

Four fertile male mice heterozygous for the mutant GDNF gene (having a hybrid 129*Sv* and C57BL/6 genetic background) were received from Dr. Heiner Westphal (Laboratory of Mammalian Genes and Development, National Institutes of Health, Bethesda, MD, USA) [8, 9]. A colony of mice was established at Monash University through matings with C57BL/6 females. All experiments were approved in advance by a Monash University animal ethics committee and were conducted in accordance with the "Australian Code of Practice for the Care and Use of Animals for Scientific Purposes."

At PN30, mice were sexed, and body weights were recorded prior to sacrifice by cervical dislocation. Tail tissue was cut for genotyping. Kidneys were then removed and decapsulated, and all visible fat and external renal vessels were removed prior to recording kidney weights. Kidneys were immersion fixed in 10% formalin for 48 hours. Kidneys used for nephron number estimation were then processed for embedding in glycolmethacrylate. For ureteric duct, kidneys were processed and embedded in paraffin.

Genotyping

All animals were routinely genotyped using the polymerase chain reaction (PCR) and the genotype subsequently confirmed by Southern blot analysis. Oligonucleotide primers (GIBCO BRL Life Technologies, Gaithersburg, MD, USA) used in the PCR assay were as published by Pichel et al [8, 9]. The specific primer sequences were as follows: p1 (5′-CCAGAGAATTCC AGAGGGAAAGGTC-3′), p2 (5′-CAGATACATCC ACACCGTTTAGCGG-3′), p3 (5′-GATCCCCTCAG AAGAACTCGT), and p4 (5′-CTGTGCTCGACGTTG TCACTG-3′). PCR amplification conditions were as follows: Samples were heated to 94°C for 5 minutes and then cycled at 94°C for 30 seconds, 55°C for 55 seconds, and 72°C for 60 seconds for 33 cycles and a final polymerase extension for 10 minutes at 72°C. The wild-type GDNF allele was detected using the p1 upstream oligonucleotide and the p2 downstream oligonucleotide, generating a 338 bp fragment. PCR amplification using the p3 and the p4 primer pairs resulted in a 567 bp amplicon detecting the mutant GDNF allele. For Southern blot analysis, the targeted allele was detected by probing a *Hin*dIII digest of tail derived DNA with a random prime-labeled neomycin phosphotransferase-specific probe (0.6 kb *Pst 1* fragment of pMC1NEO; Stratagene, La Jolla, CA, USA).

Estimating kidney volume

Kidney volume was estimated using the Cavalieri Principle [16]. Briefly, whole kidneys embedded in glycolmethacrylate were exhaustively sectioned at 20 μm. Beginning with a random start (between 1 and 10), every 10th and 11th sections were collected and stained with hematoxylin and eosin. The "10th" section of each pair was then placed on a microfiche reader and viewed at a final magnification of ×24.25. A stereological test grid (2 × 2 cm) was placed on the microfiche screen. Kidney volume was estimated using this formula:

$$V_{kid} = \Sigma P \times a(p) \times T \times 1/f \qquad \text{(Eq. 1)}$$

where V_{kid} is kidney volume, ΣP is the total number of points counted, a(p) is the area associated with each grid point, T is section thickness, and 1/f is the inverse of the section sampling fraction.

Estimating nephron number

The previously mentioned section pairs were used to estimate nephron number using the physical disector/fractionator combination [17–19]. Briefly, the section pairs were projected side by side using two microscopes modified for projection. One microscope was fitted with a motorized stage, while the other was fitted with a rotatable stage to enable section alignment. A 2 × 2 cm grid was placed over each field of view.

Grid points falling on kidney tissue (P_{kid}), glomeruli (P_{glom}), and renal corpuscle (P_{corp}) were counted. Glomeruli sampled by an unbiased counting frame in the field of view of the 10th section that were not present in the 11th section were counted. Those sampled in the 11th section that were not present in the 10th section were counted to double the efficiency of the technique. This process was repeated for each complete pair of sections. Total nephron number ($N_{glom,kid}$) was then estimated using the following equation:

$$N_{glom,kid} = 10 \times P_s/P_f \times 1/2f_a \times Q^- \quad \text{(Eq. 2)}$$

where 10 was the reciprocal of the section sampling fraction, P_s was the number of points overlying all kidney sections (complete and incomplete), P_f was the number of points overlying complete kidney sections, $1/2f_a$ was the fraction of the total section area used to count glomeruli, and Q^- was the actual number of glomeruli counted. Q^- ranged from 72 to 314 and averaged 183 per kidney.

Glomerular volume

Glomerular volume (V_{glom}) was estimated using the following:

$$V_{glom} = [V_{glom}/V_{kid}]/[N_{glom}/V_{kid}] \quad \text{(Eq. 3)}$$

where V_{glom}/V_{kid} is equivalent to P_{glom}/P_{kid}.

The combined volume of all glomeruli [$V_{glom\ (total)}$] in the kidney was estimated using:

$$V_{glom(total)} = V_{glom} \times N_{glom,kid} \quad \text{(Eq. 4)}$$

Renal corpuscle volume

Mean renal corpuscle volume (V_{corp}) was estimated using the following formula:

$$V_{corp} = [V_{corp}/V_{kid}]/[N_{glom}/V_{kid}] \quad \text{(Eq. 5)}$$

where V_{corp}/V_{kid} is equivalent to P_{corp}/P_{kid}.

The combined volume of all renal corpuscles [$V_{corp(total)}$] in the kidney was estimated using:

$$V_{corp(total)} = V_{corp} \times N_{glom,kid} \quad \text{(Eq. 6)}$$

Dolichos biflorus agglutinin histochemistry

Kidneys embedded in paraffin were exhaustively sectioned at 5 μm, and every 40th section was collected. Sections were deparaffinized and rehydrated through xylene and a series of graded ethanols immediately before use. Sections were washed in phosphate-buffered saline (PBS) and incubated at room temperature with 2% bovine serum albumin (BSA) in 0.3% triton in PBS for 30 minutes to block nonspecific binding. This and all subsequent incubations were conducted with 50 μL solutions under a glass coverslip. Slides were kept in a humidibox on a wet sponge to prevent drying. Sections were incubated for three hours at room temperature with 20 μg/mL biotinylated *Dolichos biflorus* agglutinin (DBA; Sigma-Aldrich Pty. Ltd., Castle Hill, Australia) diluted in 0.3% triton in PBS, with 1 mmol/L $CaCl_2$/$MnCl_2$/$MgCl_2$ at a 1 in 100 dilution. Following incubation, sections were rinsed twice with PBS for five minutes each to remove excess lectin. The biotinylated DBA was visualized using the Elite streptavidin/biotin amplification ABC kit (Vector Laboratories Inc., Burlingame, CA, USA). The ABC solution was applied for one hour at room temperature. Slides were then washed twice in PBS before developing the stain with 5 mg/mL diaminobenzidine activated with 0.01% H_2O_2/PBS. Sections were counterstained with hematoxylin.

Estimation of ureteric duct volume

Dolichos biflorus agglutinin-stained sections were stereologically analyzed to estimate absolute and relative (percentage) ureteric duct volume. Absolute ureteric duct volume was estimated with the Cavalieri principle. First, sections were viewed on a microfiche reader at ×24.25, and grid points (2 × 2 cm grid) overlying kidney tissue were counted. Then every 80th section was viewed at a final magnification of ×298 using a microscope modified for projection, and points overlying ureteric duct epithelium were counted. Volumes were determined by point counting of kidney tissue and of ureteric duct with an orthogonal 2 cm grid.

Statistics

Differences between estimates for heterozygous and wild-type mice were tested using an unpaired Student *t* test. Where male and female data were obtained, a two-way analysis of variance was used to detect differences between sexes and genotypes. A probability level of 0.05 or less was accepted as statistically significant. Values are mean ± SD.

RESULTS

In all cases, genotyping by PCR was confirmed by results obtained by Southern blot analysis.

As previously described, mice were born in the normal Mendelian ratio [8, 9]. Seven of 48 (14.6%) heterozygous mice were born with unilateral agenesis. At PN30, male mice were significantly larger than female littermates ($P < 0.001$). However, no difference was observed between body weights of wild-type and heterozygous mice.

The larger body weights in males reflected larger kidney weights in comparison to females ($P < 0.001$; Table 1). Kidneys from GDNF heterozygous mice showed a wide range of kidney weight and volume. Mean kidney weight and volume in heterozygous mice were approximately 25% less than in sex-matched wild-type mice ($P < 0.001$). In those heterozygous mice with a single kidney, kidney weight was greater than in the kidneys of sex-matched wild-type littermates ($P < 0.001$). Values are presented in Table 1.

Glomerular and thereby nephron number was approximately 30% less ($P < 0.01$) in heterozygous mice than in wild-type littermates (Table 2). Interestingly, nephron number in the female with a unilateral kidney was similar to values obtained for each kidney of wild-type mice. Mean glomerular and corpuscle volumes were similar in wild-type and heterozygous mice. However, the total (combined) volume of glomeruli and renal corpuscles in

Table 1. Body weights, kidney weights, and kidney volumes for male and female wild-type and heterozygous mice at postnatal day 30

	Wild-type		Heterozygous	
	Male (10)	Female (10)	Male (10)	Female (10)
Body weight *g*	22.65 ± 2.04	18.77 ± 0.73	23.14 ± 1.80	18.58 ± 1.62
Kidney weight *g*	0.158 ± 0.021	0.125 ± 0.010	0.123 ± 0.023[a]	0.093 ± 0.022[a]
			0.200 ± 0.018[a,b] (4)	0.167 ± 0.009[a,b] (3)
Kidney volume mm^3	163.15 ± 21.13	114.75 ± 16.46	119.34 ± 19.01[a]	87.11 ± 21.84[a]
				137.13 (1)[b]

The number of mice in each group is given in parentheses. All values for male mice were significantly greater than female values.
[a] Significant differences ($P < 0.001$) between wild-type and heterozygous mice.
[b] Kidney weights and volumes for mice with a single kidney; these mice are not included in the total group values

Table 2. Glomerular number and volume estimates for wild-type and heterozygous female postnatal day 30 mice

	Wild-type (10)	Heterozygous (10)	Heterozygous unilateral agenesis (1)
Glomerular number	11886 ± 1277	8573 ± 2240[a]	11163
Glomerular volume $\times 10^{-4}/mm^3$	2.174 ± 0.314	2.124 ± 0.249	2.144
Total glomerular volume mm^3	2.587 ± 0.487	1.784 ± 0.392[a]	2.393
Corpuscle volume $\times 10^{-4}/mm^3$	2.300 ± 0.312	2.280 ± 0.252	2.297
Total corpuscle volume mm^3	2.738 ± 0.503	1.920 ± 0.436[a]	2.564

The number of mice in each group is shown in parentheses.
Additional data ($N = 1$) are for a kidney of a mouse with unilateral agenesis.
[a] Significant difference when compared with wild-type mice ($P < 0.01$)

Table 3. Estimates of absolute and relative ureteric duct volumes in female wild-type and heterozygous mice

	Wild-type (10)	Heterozygous (10)	Heterozygous unilateral agenesis (1)
Absolute ureteric duct volume mm^3	2.696 ± 0.392	2.058 ± 0.284[a]	4.345
Relative ureteric duct volume %	4.040 ± 0.457	3.700 ± 0.234	4.928

Numbers in each group are shown in parentheses.
Additional data are for one heterozygous mouse with unilateral renal agenesis at PN30.
[a] $P < 0.001$

the kidney was significantly reduced in heterozygous mice ($P < 0.01$). The female mouse with a single kidney showed no obvious differences in glomerular or corpuscle volume from the wild-type mice.

Ureteric duct volume

Ureteric duct volume in heterozygous mice was 24% less than in wild-type mice ($P < 0.001$; Table 3). However, the relative volume of ureteric epithelium in heterozygous mice was similar to that in wild-type mice. Absolute and relative ureteric duct volume in the kidney of the heterozygous mouse with a single kidney was greater than the mean wild-type values.

DISCUSSION

Branching morphogenesis during embryonic and/or postnatal development ultimately establishes the architectural pattern of many epithelial organs, including the lung, prostate gland, mammary gland, salivary gland, and kidney. Clinically, the pattern of branching morphogenesis in the kidney is a major determinant of nephron endowment. After successive generations of branching, the normal human kidney can contain between 300,000 and over 1 million nephrons [17]. Reduced nephron endowment has been associated with the development of essential hypertension, chronic renal failure, and the long-term success of renal allografts [20–22]. Ureteric duct branching morphogenesis is therefore a critical event in kidney development and is thought to be a crucial factor in determining nephron number.

The expression of GDNF and its receptors, in addition to the results of gene ablation studies, indicates that GDNF plays a significant role in mediating the inductive event that regulates renal branching morphogenesis. In addition, the loss of only one allele is sufficient to cause a significant renal phenotype. Since nephron initiation only occurs at the tips of ureteric branches, the aim of the present study was to determine whether the nephron number in GDNF heterozygous mice is reduced.

Although body weights of GDNF heterozygous mice

and wild-type mice were similar, heterozygous kidneys were approximately 25% smaller than wild-type kidneys. Some kidneys also showed an abnormal shape, indicating possible defects in the regulation of branching. Estimates for nephron number identified a 30% decrease in nephron endowment in heterozygous GDNF mice. However, although there were fewer nephrons, there were no indications of any compensatory glomerular hypertrophy at PN30. A reduction in glomerular number without compensatory glomerular hypertrophy presumably decreases the surface area of glomerular capillaries per kidney, although we did not measure this parameter in the present study. Glomerular hypertrophy has been associated with development of glomerulosclerosis in both animals and humans, leading to further glomerular loss.

Glomerular number varied widely between heterozygous GDNF mice from a minimum of 3542 to a maximum of 10833. This variation may reflect altered branching morphogenesis, possibly because of regional variations in endogenous GDNF levels in heterozygous animals.

It is theoretically possible that the reduced nephron endowment in GDNF heterozygous mice is the result of retarded nephron induction and/or rate of nephron development. However, this is considered extremely unlikely because the kidneys of the heterozygous mice contained no nephrogenic zone, mesenchymal condensates, vesicles, commas, or s-shaped bodies. Moreover, PN30 was chosen because this is approximately three weeks after the cessation of nephron induction in wild-type mice.

The reduced absolute volume of the ureteric duct in heterozygous GDNF mice as well as the fact that the relative volume was not significantly different suggests that the observed reduction in nephron endowment is most likely due to reduced ureteric duct branching morphogenesis. However, kidney weight and volume for a single mouse with a unilateral kidney was larger than values obtained for wild-type mice. Ureteric duct volume in this kidney was also greater than wild-type kidneys. However, this increase in size, presumably in compensation for the absent kidney, was not accompanied by an increase in the number of nephrons greater than in wild-type mice. It is possible that epithelial cell hyperplasia or hypertrophy rather than branching may have resulted in the increase in ureteric duct volume. In addition, it is not known whether the size of this kidney was different to wild-types at birth or as a result of physiological hypertrophy was considerably larger at PN30.

Available evidence from in vitro studies suggests that other members of the GDNF family, such as neurturin and persephin, may also play a role in the regulation of ureteric branching morphogenesis. Both neurturin and persephin are able to partially restore, dose dependently, collecting duct arborization in kidneys depleted of their sulfated glycosaminoglycans [23, 24]. Applied to agarose beads, neurturin induces supernumerary buds from the Wolffian duct and causes the closest branches to expand to an abnormally large diameter [23].

Although neurturin and persephin show in vitro roles in metanephric development, their true in vivo roles remain unclear. However, considering that not all homozygous GDNF mutants show complete bilateral absence of ureteric duct development, the other GDNF family members, as well as possible pathways independent of RET [25–28], may provide a degree of redundancy in the regulation of ureteric duct branching morphogenesis in vivo.

It is now commonly accepted that GDNF secreted by the mesenchyme is a major signal for the outgrowth of the ureteric bud from the Wolffian duct and plays a significant role in early ureteric branching morphogenesis. The regulation of nephron endowment is believed to be controlled by two major factors, first branching morphogenesis and second nephron induction. The molecular regulation of branching morphogenesis, as well as nephron induction, is achieved through a complex interaction between key transcription factors, growth factors, their receptors, and the extracellular matrix [29–32]. Furthermore, the spatial and temporal expression of these key factors ultimately regulates the shape and degree of branching, with the induction of nephrons at the tips of these branches generating the architectural pattern of the metanephric kidney.

In conclusion, the present results indicate that GDNF plays a significant, but partial role in the determination of nephron number. Further analysis of the branching pattern in embryonic heterozygous GDNF mice may indicate more precise roles that GDNF plays in the regulation of branching morphogenesis and therefore nephron endowment.

ACKNOWLEDGMENTS

This work was supported by the Australian Research Council (ARC). Luise Cullen's Ph.D. scholarship is funded by the Australian Kidney Foundation. The authors also thank Jim Massalas (Department of Medicine, Monash University, Monash, Australia) for his technical help with the Southern blots. John Drago is a Logan Research Fellow at Monash University.

Reprint requests to John F. Bertram, Ph.D., Department of Anatomy and Cell Biology, Monash University, Clayton, Victoria, Australia, 3800. E-mail: john.bertram@med.monash.edu.au

REFERENCES

1. Hellmich HL, Kos L, Cho ES, *et al:* Embryonic expression of glial cell-line derived neurotrophic factor (GDNF) suggests multiple developmental roles in neural differentiation and epithelial-mesenchymal interactions. *Mech Dev* 54:95–105, 1996
2. Suvanto P, Hiltunen JO, Arumae U, *et al:* Localization of glial cell line-derived neurotrophic factor (GDNF) mRNA in embryonic rat by in situ hybridization. *Eur J Neurosci* 8:816–822, 1996
3. Pachnis V, Mankoo B, Costantini F: Expression of the c-ret proto-oncogene during mouse embryogenesis. *Development* 119: 1005–1017, 1993
4. Sainio K, Suvanto P, Davies J, *et al:* Glial-cell-line-derived neurotrophic factor is required for bud initiation from ureteric epithelium. *Development* 124:4077–4087, 1997
5. Schuchardt A, D'Agati V, Larsson-Blomberg L, *et al:* Defects in

the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor RET. *Nature* 367:380–383, 1994
6. Schuchardt A, D'Agati V, Pachnis V, *et al:* Renal agenesis and hypoplasia in ret-k$^-$ mutant mice result from defects in ureteric bud development. *Development* 122:1919–1929, 1996
7. Moore MW, Klein RD, Farinas I, *et al:* Renal and neuronal abnormalities in mice lacking GDNF. *Nature* 382:76–79, 1996
8. Pichel JG, Shen L, Sheng HZ, *et al:* Defects in enteric innervation and kidney development in mice lacking GDNF. *Nature* 382:73–76, 1996
9. Pichel JG, Shen L, Sheng HZ, *et al:* GDNF is required for kidney development and enteric innervation, in *Cold Spring Harbor Symposia on Quantitative Biology (445457)*, Cold Spring Harbor, Cold Spring Harbor Laboratory Press, 1996
10. Sanchez MP, Silos-Santiago I, Frisen J, *et al:* Renal agenesis and the absence of enteric neurons in mice lacking GDNF. *Nature* 382:70–73, 1996
11. Cacalano G, Farinas I, Wang L, *et al:* GFRα-1 is an essential receptor component for GDNF in the developing nervous system and kidney. *Neuron* 21:53–62, 1998
12. Enomoto H, Araki T, Jackman A, *et al:* GFR alpha-1 deficient mice have deficits in the enteric nervous system and kidneys. *Neuron* 21:317–324, 1998
13. Vega QC, Worby CA, Lechner MS, *et al:* Glial cell line-derived neurotrophic factor activates the receptor tyrosine kinase RET and promotes kidney morphogenesis. *Proc Natl Acad Sci USA* 93:10657–10661, 1996
14. Pepicelli CV, Kispert A, Rowitch DH, *et al:* GDNF induces branching and increased cell proliferation in the ureter of the mouse. *Dev Biol* 192:193–198, 1997
15. Towers PR, Woolf AS, Hardman P: Glial cell line-derived neurotrophic factor stimulates ureteric bud outgrowth and enhances survival of ureteric bud cells *in vitro. Exp Nephrol* 6:337–351, 1998
16. Gundersen HJG, Bagger P, Bendtsen TF, *et al:* The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *Acta Pathol Microbiol Immunol Scand* 96:857–881, 1988
17. Nyengaard JR, Bendtsen TF: Glomerular number and size in relation to age, kidney weight and body surface in normal man. *Anat Rec* 232:194–201, 1992
18. Bertram JF, Soosaipillai MC, Ricardo SD, *et al:* Total numbers of glomeruli and individual glomerular cell types in the normal rat kidney. *Cell Tissue Res* 270:37–45, 1992
19. Bertram JF: Analyzing renal glomeruli with the new stereology. *Int Rev Cytol* 161:111–172, 1995
20. Brenner BM, Garcia DL, Anderson S: Glomeruli and blood pressure: Less of one more of the other? *Am J Hypertens* 1:355–347, 1988
21. Brenner BM, Milford EL: Nephron underdosing: A programmed cause of chronic renal allograft failure. *Am J Kidney Dis* 21:66–72, 1993
22. Brenner BM, Chertow GM: Congenital oligonephropathy and the aetiology of adult hypertension and progressive renal injury. *Am J Kidney Dis* 23:171–175, 1994
23. Davies JA: Neurturin: An autocrine regulator of renal collecting duct development. *Dev Genet* 24:284–292, 1999
24. Milbrandt J, de Sauvage FJ, Fahrner TJ, *et al:* Persephin, a novel neurotrophic factor related to GDNF and neuturin. *Neuron* 20:245–253, 1998
25. Poteryaev D, Titievsky A, Sun YF, *et al:* GDNF triggers a novel ret-independent Src kinase family-coupled signaling via a GPI-linked GDNF receptor alpha1. *FEBS Lett* 463:63–66, 1999
26. Trupp M, Raynoschek C, Belluardo N, *et al:* Multiple GPI-anchored receptors control GDNF-dependent and independent activation of the c-ret receptor tyrosine kinase. *Mol Cell Neurosci* 11:47–63, 1998
27. Trupp M, Scott R, Whittemore SR, *et al:* Ret-dependent and -independent mechanisms of glial cell line-derived neurotrophic factor signaling in neuronal cells. *J Biol Chem* 274:20885–20894, 1999
28. Airaksinen MS, Titievsky A, Saarma M: GDNF family neurotrophic factor signaling: Four masters, one servant? *Mol Cell Neurosci* 13:313–325, 1999
29. Horster MF, Braun GS, Huber SM: Embryonic renal epithelia: Induction, nephrogenesis, and cell differentiation. *Physiol Rev* 79:1157–1191, 1999
30. Burrow C: Regulatory molecules in kidney development. *Pediatr Nephrol* 14:240–253, 2000
31. Clark AT, Bertram JF: Advances in renal development. *Curr Opin Nephrol Hypertens* 9:247–251, 2000
32. Kuure S, Vuolteenaho R, Vainio S: Kidney morphogenesis: Cellular and molecular regulation. *Mech Dev* 92:31–45, 2000